UDC 547.457.1'118.5'117.12 : 577.15

80. **Tohru Ueda** : Studies on Coenzyme Analogs. IV.[*1] Syntheses and Properties of β-D-Glucose 1-Phosphoramidate.

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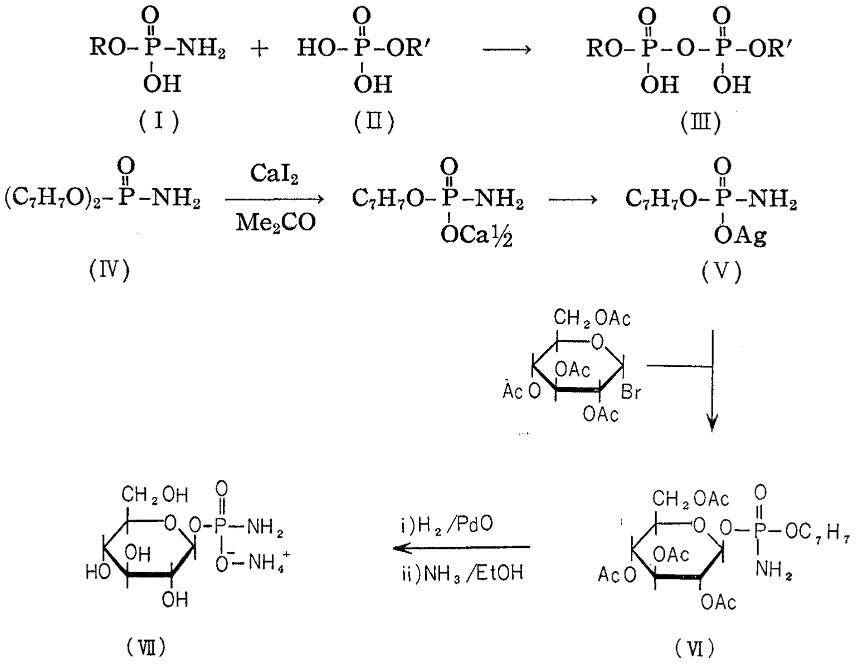
It is well known that a key point of the synthesis of nucleotide coenzymes lies in the pyrophosphate-bond synthesis. At present, the phosphoramidate method developed by Todd[1)] and Khorana[2)] is the best way to synthesize asymmetrical pyrophosphates. Todd[1)] utilized this method for synthesis of ADP[*3] (III : R=H, R'=adenosin-5'-yl) from benzyl phosphoramidate (I : R=C_7H_7-) and AMP (II : R=adenosin-5'-yl) while Khorana[2)] obtained UDPG (III : R=uridin-5'-yl, R'=α-D-glucos-1-yl) and FAD (III : R=adenosin-5'-yl, R'=riboflavin-5'-yl) in a good yield from corresponding nucleoside 5'-phosphoramidates.

In the present work, attempt was made to synthesize β-D-glucose 1-phosphoramidate (VII) as one of the starting materials for the synthesis of UDP-β-glucose and other unnatural nucleotide analogs, expecting that the sugar phosphoramidate would form the pyrophosphate bond by phosphorolysis similar to other phosphoramidates.



$$RO-\overset{O}{\underset{OH}{P}}-NH_2 + HO-\overset{O}{\underset{OH}{P}}-OR' \longrightarrow RO-\overset{O}{\underset{OH}{P}}-O-\overset{O}{\underset{OH}{P}}-OR'$$

(I) (II) (III)

$$(C_7H_7O)_2-\overset{O}{P}-NH_2 \xrightarrow[Me_2CO]{CaI_2} C_7H_7O-\overset{O}{\underset{OCa\frac{1}{2}}{P}}-NH_2 \longrightarrow C_7H_7O-\overset{O}{\underset{OAg}{P}}-NH_2$$

(IV) (V)

(VII) (VI)

Dibenzyl phosphoramidate[3)] (IV) was refluxed with calcium iodide in acetone to afford calcium benzyl phosphoramidate. This calcium salt was converted to its silver salt (V) and reacted with acetobromoglucose in benzene for 90 minutes. The ester (VI) thus obtained was a vitreous matter and gave a solid ammonium salt of (VII) by successive hydrogenation in dehyd. ethanol and deacetylation with ammonia in ethanol.

[*1] Part III : This Bulletin, 8, 455(1960).

[*2] Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido (上田 亨).

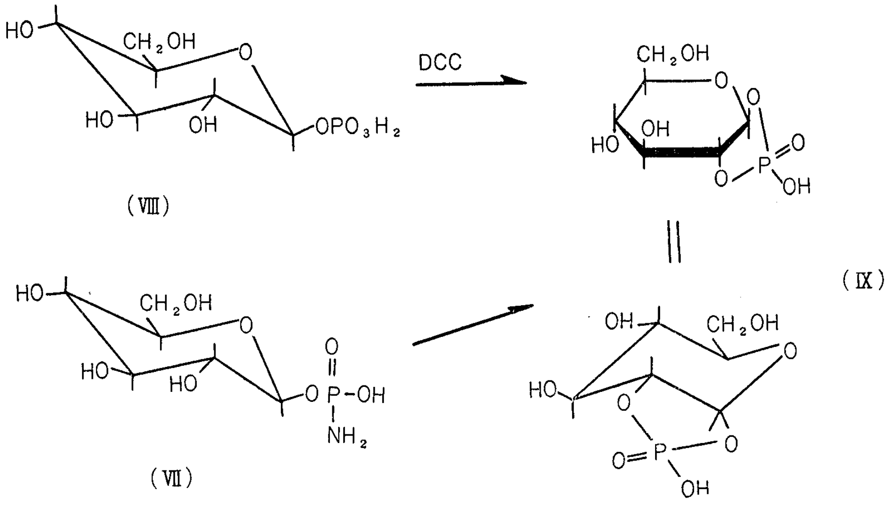
[*3] Following abbreviations are used : AMP, adenosine 5'-phosphate; ADP, adenosine 5'-diphosphate; UMP, uridine 5'-phosphate; UDP, uridine 5'-diphosphate; UDPG, uridine diphosphate glucose; FAD, flavin adenine dinucleotide; DCC, dicyclohexyl carbodiimide.

1) A.R. Todd, *et al.* : J. Chem. Soc., **1957**, 1497.

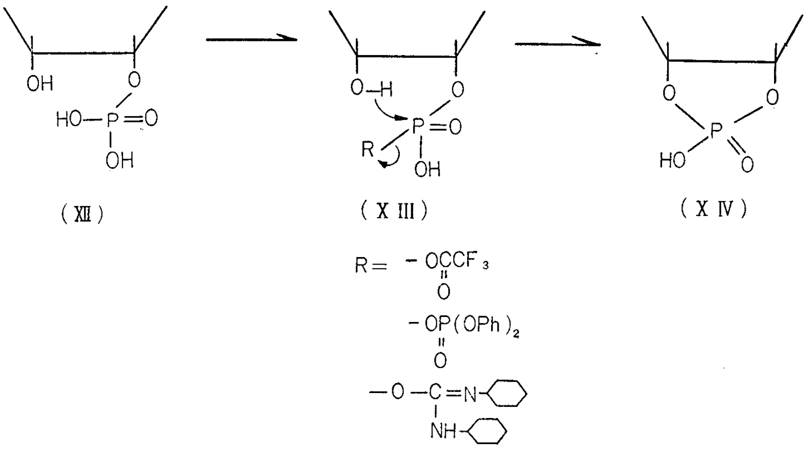
2) J.G. Moffatt, H.G. Khorana : J. Am. Chem. Soc., 80, 3756(1958).

3) A.R. Todd, *et al.* : J. Chem. Soc., **1945**, 660.

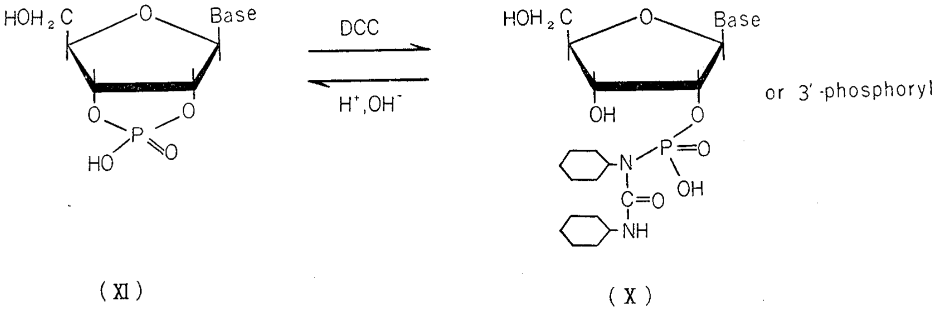
The conformation of its glycosidic linkage was assigned as β-form, estimated by the specific rotation value and acid hydrolysis of the amidate to glucose and inorganic phosphate through β-D-glucose 1-phosphate (Table III). This observation presents one more example to the findings[4] that acetobromoglucose gives α-glucose 1-phosphate by phosphorylation with trisilver phosphate and silver diphenylphosphate, and gives β-anomer with monosilver phosphate and silver dibenzylphosphate, the regularity between glycosidic configuration and the type of phosphorylating agents being obscure.



(VIII) (VII) (IX)



(XII) (XIII) (XIV)



(XI) (X)

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[4] A. B. Foster, W. G. Overand : Quart. Revs., **11**, 61(1957).

The barium salt of the amidate (VII) thus synthesized was negative to the Nessler test which became positive after being heated at 100° in 0.1*N* hydrochloric acid for 2 minutes. When the free amidate was heated in water it gave a positive test. This shows the instability of P–N bond in acid medium and is a proof for the structure of the phosphoramidate to be (VII). The hydrolytic behaviors of (VII) toward acid and alkali were further investigated by paper chromatography (Table I). Decomposition of the amidate to β-D-glucose 1-phosphate (VIII) was observed after 30 minutes in 0.1*N* hydrochloric acid at room temperature, was complete after 28 hours, and a small amount of inorganic phosphate was detected together. When treated with 0.1*N* sodium hydroxide solution, it was decomposed slightly after 28 hours and in 1*N* ammonia solution no decomposition was observed. To examine the pyrophosphate-forming tendency of this amidate (VII), reaction of its amine salt with phosphoric acid, benzyl dihydrogenphosphate, and UMP was attempted in several solvents. Contrary to expectations, formation of pyrophosphate compounds did not occur in any of the cases and, in the case of UMP (precise investigation was not undertaken in other cases), the only product detected on paper chromatogram was a sugar phosphate with higher Rf value. This substance was paper chromatographically identified with the synthetic β-D-glucose 1,2-cyclic phosphate (IX) obtained from the reaction of β-D-glucose 1-phosphate (VIII) and DCC in the presence of an amine.5) From this evidence, it is concluded that the amidate (VII) underwent intramolecular cyclization to the 1,2-cyclic phosphate (IX) in anhydrous basic medium.

The reaction observed this time could be compared with that for N-phosphorylurea compound6) (X) produced by the reaction of nucleoside 2′,3′-cyclic phosphate with DCC. Thus, (X) is easily cyclized to the 2′,3′-cyclic phosphate (XI) in an acid or alkaline medium.

These compounds, (VII) and (X), commonly have a hydroxyl group esterified by a phosphoryl group involving highly activated P–N linkage and another hydroxyl group which is situated at the carbon atom adjacent to the phosphate-bearing one in a conformation other than *trans*-axial orientation. In such a structural moiety, the phosphorus center should most easily be attacked intramolecularly by the neighboring hydroxyl group to form a cyclic phosphate. Several reactions in which a similar cyclization of phosphoryl group by the nucleophilic attack of vicinal hydroxyl group have hitherto been reported. Thus, nucleoside 2′(or 3′)-phosphate6~8) and 1-phosphate5) of glucose, arabi-

Table I. Hydrolysis of β-D-Glucose 1-Phosphoramidate by Acid or Alkali

Solvent	Period at room temp. (hr.)	Product	
		Major	Minor
0.1*N* HCl	0.5	β-G1P-NH_2	β-G1P
	28.0	β-G1P	iP
	48.0	β-G1P	iP
	240.0	iP	β-G1P
0.1*N* NaOH	0.5	β-G1P-NH_2	
	28.0	β-G1P-NH_2	β-G1P
	48.0	β-G1P-NH_2	β-G1P
0.1*N* NH_4OH	240.0	β-G1P-NH_2	

β-G1P-NH_2, β-D-glucose 1-phosphoramidate; β-G1P, β-D-glucose 1-phosphate; iP, inorganic phosphate.

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5) H.G. Khorana, G.M. Tener, R.S. Wright, J.G. Moffatt: J. Am. Chem. Soc., **79**, 430(1957). The formation of α- and β-glucose 1,2-cyclic phosphate by the reaction of α- and β-glucose 1-phosphate and DCC is reported but the precise synthetic procedure and properties of the cyclic phosphate are not described in this report, since the present author synthesized this substance as described in Experimental part.

6) C.A. Dekker, H.G. Khorana: J. Am. Chem. Soc., **76**, 3522(1954).

7) D.M. Brown, D.L. Magrath, A.R. Todd: J. Chem. Soc., **1952**, 2708.

8) A.M. Michelson: Chem. & Ind. (London), **1958**, 70.

Table II. Rf values in Paper Chromatography

Solvent system / Substance	I	II	III
Inorganic phosphate	0.20	0.14	0.08
β-D-Glucose 1-phosphate	0.44	0.19	0.13
β-D-Glucose 1-phosphoramidate	0.60	0.37	0.42
β-D-Glucose 1,2-cyclic phosphate	0.78	—	0.56

Solvent system : I. iso-PrOH:conc. NH_4OH:H_2O(7:1:4); II. EtOH: 0.5N $AcONH_4$(5:2); III. Methylcellosolve:MeCOEt:3N NH_4OH(7:2:3, saturated with boric acid).

Detection : Sugar by periodate-benzidine spray,[10] phosphate by $HClO_4$-molybdate spray.[11]

nose, and xylose (XII), when activated by a reagent such as diphenyl phosphorochloridate,[8] tetraphenyl pyrophosphate,[8] trifluoroacetic anhydride,[7] or DCC,[5,6] give the corresponding cyclic phosphates (XIV) through the proposed intermediate (XIII). The observed result for β-D-glucose 1-phosphoramidate (VII) which did not condense with UMP in an anhydrous basic medium to give the expected pyrophosphate compound but underwent intramolecular cyclization could be explained by the above discussion.

A question as to whether α-D-monnose 1-phosphoramidate, in which the phosphoryl group and the neighboring hydroxyl group are in *trans*-axial conformation, would be converted to the pyrophosphate or not, will be answered by further experiments.

Table III. Change of Specific Rotation during Degradation of β-D-Glucose 1-Phosphoramidate in 0.1N HCl

Time (hr.) / $[\alpha]^{10}_{589}$ calcd. as	0	1	2	6	20	72	500
β-G1P-NH_2	+2.50°	+5.69°	+6.15°	+7.97°	+10.48°	+15.2°	+29.6°
β-G1P				+10.7°	+14.0°	+20.4°	
Glucose							+57.5°
Main product	β-G1P-NH_2			β-G1P	β-G1P	β-G1P	iP, Glucose
Control $[\alpha]^{10}_{589}$ (H_2O)	+3.49°	+4.22°	+4.22°	+2.93°	+2.30°	+2.19°	+3.87°

β-G1P-NH_2, β-D-glucose 1-phosphoramidate; β-G1P, β-D-glucose 1-phosphate; iP, inorganic phosphate. Main product was determined by paper chromatography.

Experimental

Silver Benzyl Phosphoramidate (V)—Dibenzyl phosphoramidate (IV) (3.9 g.) and CaI_2 (2.4 g.) in Me_2CO (50 cc.) were refluxed for 6 hr. When cool, the precipitated calcium salt was collected, washed thoroughly with Me_2CO until the filtrate became colorless, and dried (2.3 g.). *Anal.* Calcd. for $C_7H_9O_3NCa_{1/2}P$: N, 6.79. Found : N, 6.59.

The Ca salt was dissolved in boiling water, cooled rapidly, and an aqueous solution of $AgNO_3$ (1.1 moles) was added in the dark. White precipitate formed was collected, washed with EtOH and Et_2O, and dried *in vacuo* in darkness.

Ammonium β-D-Glucose 1-Phosphoramidate (VII)—Benzene solution (40 cc.) of acetobromoglucose (6 g.) and freshly prepared silver benzylphosphoramidate (8 g.) was heated at 50° for 1 hr. and then refluxed for 30 min. with continuous stirring in the darkness. The precipitate was filtered off and benzene solution was washed successively with water, 2% ice-cold $NaHCO_3$ solution, and water, and dried over Na_2SO_4. Faint yellow gum (VI) which was obtained by evaporation of benzene was taken up in dehyd. EtOH (50 cc.) and hydrogenated over PdO catalyst. Rapid uptake of H_2 occurred and ceased within 30 min. After removal of the catalyst by filtration, EtOH solution was saturated with NH_3 at 0° and allowed to stand overnight at room temperature. White precipitate was collected, washed with dehyd. EtOH and Et_2O, and dried *in vacuo* (2.5 g). It was very hygroscopic powder but

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9) M. L. Wolfrom, *et al.* : J. Am. Chem. Soc., **64**, 23 (1942).

10) M. Viscontini, O. Hoch, P. Karrer : Helv. Chim. Acta, **38**, 642(1955).

11) C. S. Hanes, F. A. Isherwood : Nature, **164**, 1107(1949).

paper chromatographically homogeneous. $[\alpha]^{10}_{589}$ +2.9°(c=0.875 in H_2O). *Anal.* Calcd. for $C_6H_{17}O_8N_2P\cdot 4H_2O$: P, 8.92. Found : P, 9.09. Barium salt : $[\alpha]^{14}_{589}$ +5.0°(c=2.305 in H_2O). *Anal.* Calcd. for $C_6H_{13}O_8NBa_{1/2}P$: C, 22.10; H, 3.98. Found : C, 22.00; H, 4.25. It gave negative reaction with Nessler's reagent but after keeping at 100° in 0.1*N* HCl, the reaction became positive.

When the ammonium salt was passed through a column of Amberlite IR-120, it still showed negative reaction against Nessler's reagent, but on heating at 100° for 2 min., it became positive. Rf values of paper chromatography of amidate and related compounds are shown in Table II and hydrolytic behavior of the amidate toward acid and alkali is shown in Table I which was detected by paper chromatography in solvent system III (Table II).

Change of Optical Rotation during the Hydrolysis of β-D-Glucose 1-Phosphoramidate in 0.1*N* Hydrochloric Acid—Ammonium β-D-glucose 1-phosphoramidate tetrahydrate was dissolved in 0.1*N* HCl and changes of specific rotation was measured (Table III). This shows that when amidate was decomposed largely to glucose 1-phosphate, its specific rotation as glucose phosphate was +10.7° to +14.0°, which was in agreement with the specific rotation of β-anomer calculated from its dibrucinate as +12.9.[9] Specific rotation of α-isomer is +120°.

β-D-Glucose 1,2-Cyclic Phosphate (IX)—Dibrucinium salt of β-D-glucose 1-phosphate[9] (VIII) (10 mg.) was dissolved in formamide (0.5 cc.), and cyclohexylamine (0.1 cc.) and DCC (0.1 g.) in *tert*-BuOH (1 cc) were added to the solution. The mixture was heated at 60° for 8 hr., precipitated dicyclohexylurea was collected, and washed with water. The combined solution was concentrated under a reduced pressure to remove *tert*-BuOH and extracted with Et_2O. Aqueous layer was concentrated and the residual solution was used directly for paper chromatography as β-D-glucose 1,2-cyclic phosphate (Table II).

Reaction of the Amidate with Several Phosphates—Ammonium β-D-glucose 1-phosphoramidate was converted to its pyridinium, triethylammonium, or brucinium salt and mixed with H_3PO_4, benzyl dihydrogenphosphate, or UMP in pyridine or pyridine-water. The mixed solution was allowed to stand for 8 days at room temperature and applied to paper chromatography. No reaction occurred in any of the cases, but, in the case of UMP and brucinium β-D-glucose 1-phosphoramidate in anhyd. pyridine, a spot with higher Rf value (0.56 in solvent system III) was detected on paper chromatogram. It was undistinguishable from β-D-glucose 1,2-cyclic phosphate in paper chromatography.

The author expresses his gratitude to Prof. Y. Mizuno of this University for his guidance throughout the course of this work. He is indebted to Mr. K. Narita for elemental analyses. A part of the expenses for this work was defrayed by the Grant-in-Aid for Scientific Research from the Ministry of Education for 1959, to which the author's thanks are due.

Summary

β-D-Glucose 1-phosphoramidate was synthesized by the reaction of silver benzyl phosphoramidate and acetobromoglucose, and successive hydrogenation and deacetylation. The structure of (VIII) was confirmed by acid hydrolysis and optical rotation. The amidate had no ability to form pyrophosphate by phosphorolysis with another phosphate, as the other phosphoramidates do, but afforded the β-D-glucose 1,2-cyclic phosphate, due to the specific configuration which was favorable to intramolecular cyclization. Some discussion was made about this cyclization.

(Received November 5, 1959)